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The overall objective of this study is to improve our understanding of the lipid dependent biochemical processes involved in breast cancer development, in order to develop more effective diet based cancer prevention strategies. We have shown previously that alterations in dietary lipid can significantly influence the development of mammary tumors. The experiments in this project are designed to explore to what extent, if any, dietary lipid manipulations may influence the expression of IGFs and their receptors in two different mammary tumor models. During the 1995-96 grant year, we completed studies in R3230AC tumor development in F-344 rats and NMU induced tumorigenesis in Sprague Dawley rats. The results to date have shown no correlation between serum IGF levels and the dietary fat composition. Comparative studies on the mRNA content of these tumors for the IGFs and their receptors are ongoing at present.

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FOREWORD

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William T. Carter 8/12/97

PI - Signature Date

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INTRODUCTION:

The following report summarizes the effort expended during the second year of grant funding. During this time two individual experiments were carried out; an initial study involving R3230AC transplanted tumors and a second one evaluating the effects of dietary lipids on NMU induced mammary tumors. A third study was initiated during the period of this report and was ongoing in Oct. 1996.

BODY:

Our initial attempt to induce mammary tumorigenesis in female F-344 rats with NMU was unsuccessful. The F-344 rats in the different diet groups did not develop sufficient tumors for group analysis. The reasons for this lack of success is unclear in light of the published work of others. However, given this disappointing result, it was concluded that further NMU tumor induction should be done in Sprague Dawley rats where we had previously proven success.

The study with F-344 rats treated with R3230AC tumor transplants was successfully completed. In this experiment, 28 week old female rats received two tumor implants in their mammary fat pads. They were then divided into 4 diet groups: 20%CO, 5%CO, 4%FO+1%CO, and 19%FO+1%CO. The tumors grew rapidly in all rats and they were killed 21 days following tumor transplantation. There tumors were removed, weighed, and their membranes analyzed for their fatty acid profiles. The fatty acid profiles are shown in figure 1, and demonstrate that the different diets did induce important alterations in their tumor membrane fatty acid compositions. Table 1 presents the weights of the tumors from the different diet groups.

Figure 1.

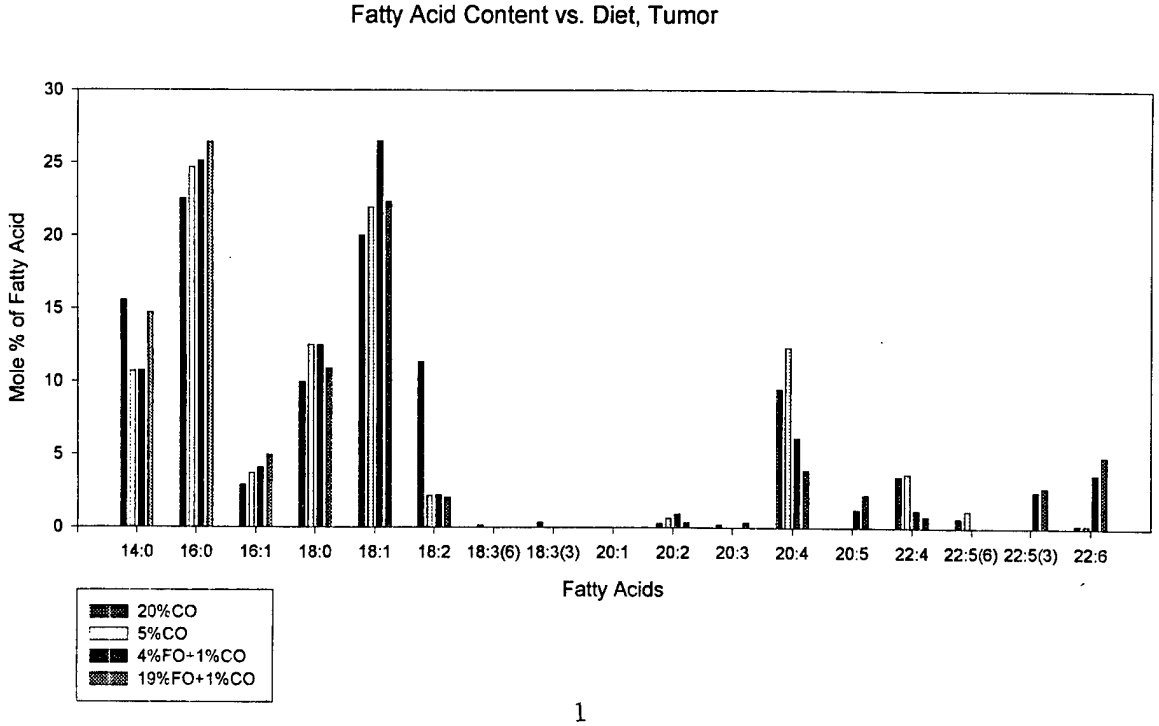


Table 1.

R3230AC Tumor Burden
(Mean± S.E.)

Diet Group	20% CO	5% CO	4%FO+1%CO	19%FO+1%CO
n	4	5	4	6
Tumor Wt.	4.617	4.806	4.220	3.00
	± 0.396	1.358	1.137	0.790

These results indicated that there was a trend toward a reduced tumor burden in the rats fed a high fish oil diet relative to the high corn oil diet, but this difference was not statistically significant. The remaining tumor tissue is currently stored at -70 deg. C, so it can be analyzed for IGF and IGF receptor message at a later time.

In October 1995 we conducted an experiment using the n-methyl nitrosourea (NMU) induced mammary tumor model, where female Harlan Sprague Dawley rats received an IV infusion of aqueous NMU (5mg/100g body weight) at 50 days of age. They subsequently were placed on their respective semisynthetic diets and monitored for tumor development. The diets were prepared in the vivarium diet kitchen using analyzed ingredients of uniform quality. The corn oil was obtained commercially(ICN/Teklad) and the menhaden oil was obtained from the NIH/NOAA biochemical test material program(Southeast Fisheries Science Center, Charleston SC). The oils were adjusted with the antioxidants alpha tocopherol, gamma tocopherol, and tertiary butylhydroquinone in order to maintain equivalent levels in both oils. The diets were equicaloric in the respective high and low fat diet groups, and appropriate adjustments were made for protein, mineral, and vitamin content. The time of first palpable tumor occurrence was recorded, and when the rats developed tumors approximately 2 cm in diameter they were killed, their sera collected, and autopsied. The tumor latent period was calculated as the time from carcinogen administration until death, and the tumor burden was the total weight of the tumor tissue obtained at autopsy. The sera and tumor tissue are stored at -70 degrees centigrade until used for specific assay. The fatty acids profiles of microsomal membranes from representative tumors were assessed by gas chromatography. Serum IGF-1 was measured by using a radioimmunoassay kit (Nichols Institute Diagnostics). All serum samples from each experiment were evaluated in a single batch analysis. Studies to measure the mRNAexpression of the IGFs and their respective receptors are currently in progress.

The results of fatty acid profile analyses of the tumor membranes of these animals are presented in figures 2 and 3. The data on tumorigenesis and serum IGF levels from each of the diet groups is presented in table 2, and figures 4-7. All values are presented as mean values. The number [n] or individual analyses is that of the group unless specifically noted.

Figure 2.

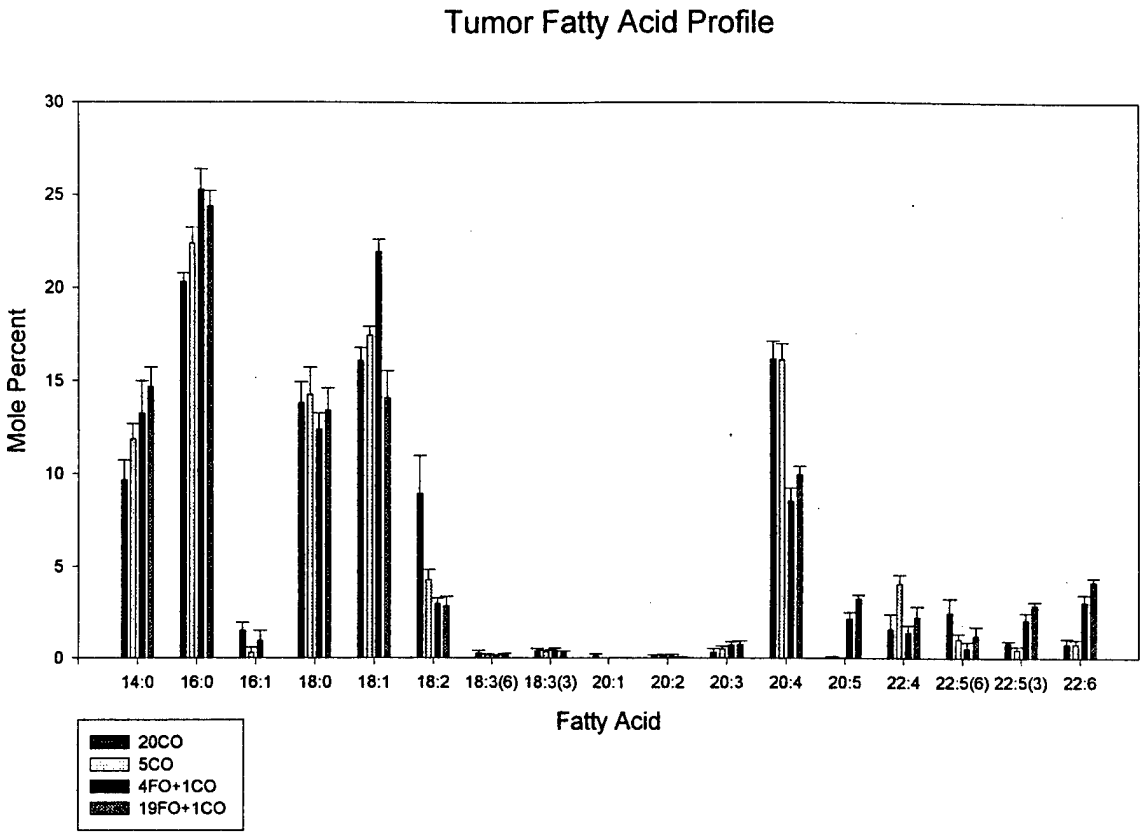


Figure 3.

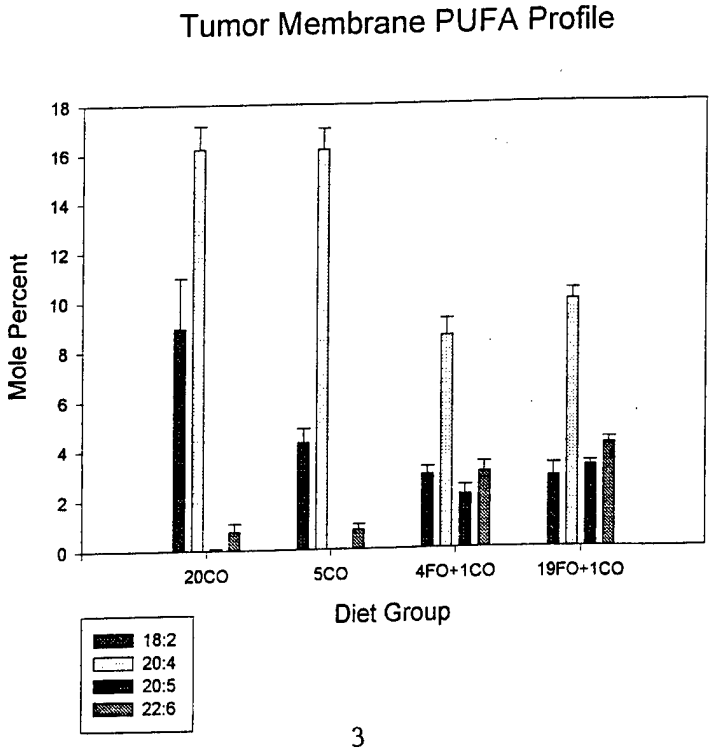
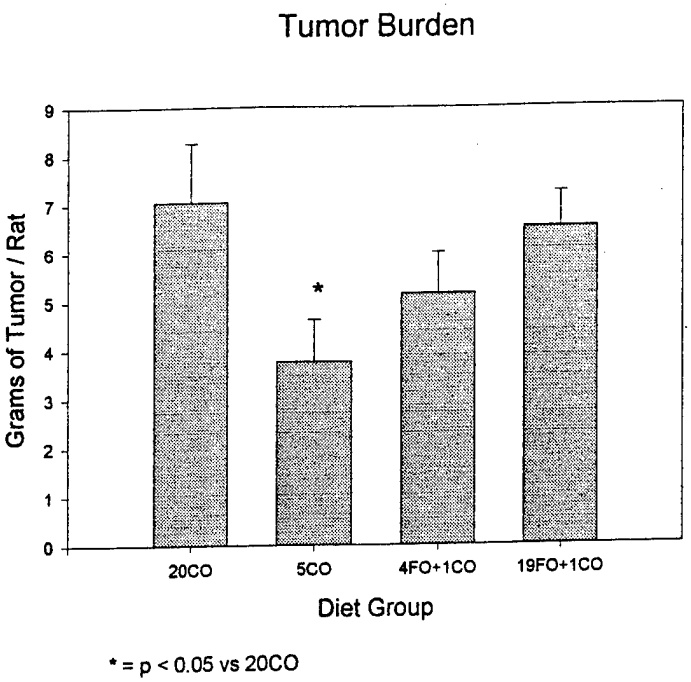


Figure 6.



* = p < 0.05 vs 20CO

Figure 7.

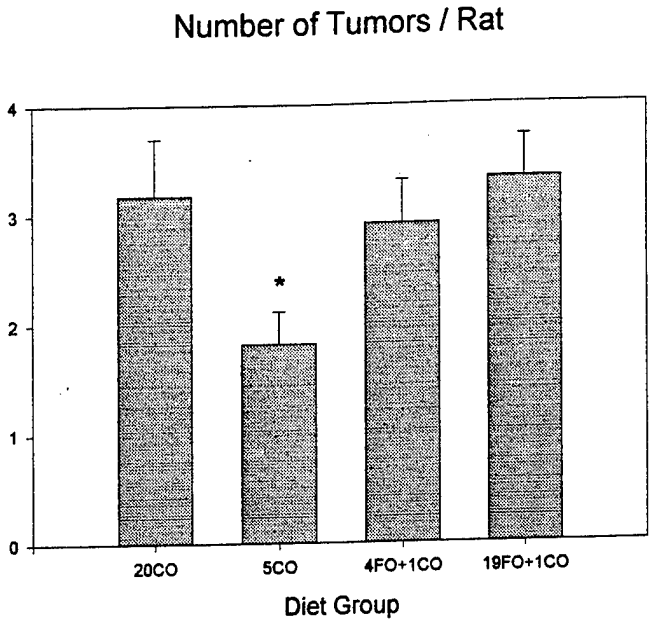


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Figure 7.

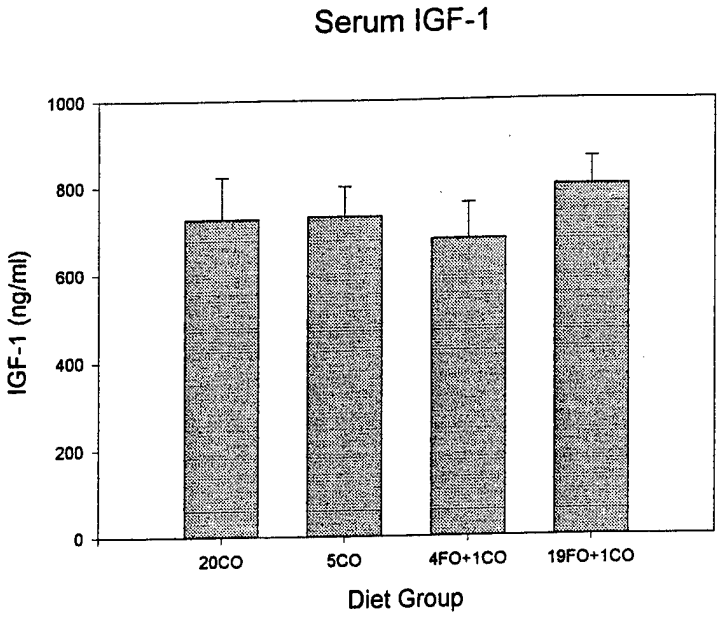


Table 2.

NMU Induced Tumorigenesis
(Mean ± S.E.)

Diet Group	[n]	First Tumor Appearance (days)	Tumor Latency (days)	Tumor Burden (grams)	Tumor Number	Serum IGF-1 ng/mL
20%CO	11	78.64 ± 8.72	117.36 9.62	7.07 4.01	3.18 0.52	726.40 96.98
5%CO	11	85.18 ± 8.27	116.36 8.97	3.77 2.88	1.82 0.30	731.80 68.51
4%FO+1%CO	12	101.00 ± 7.91	122.83 6.34	5.17 2.94	2.92 0.40	678.60 82.23
19%FO+1%CO	12	78.90 ± 4.50	111.66 6.69	6.53 2.51	3.33 0.38	802.40 62.86

Figure 4.

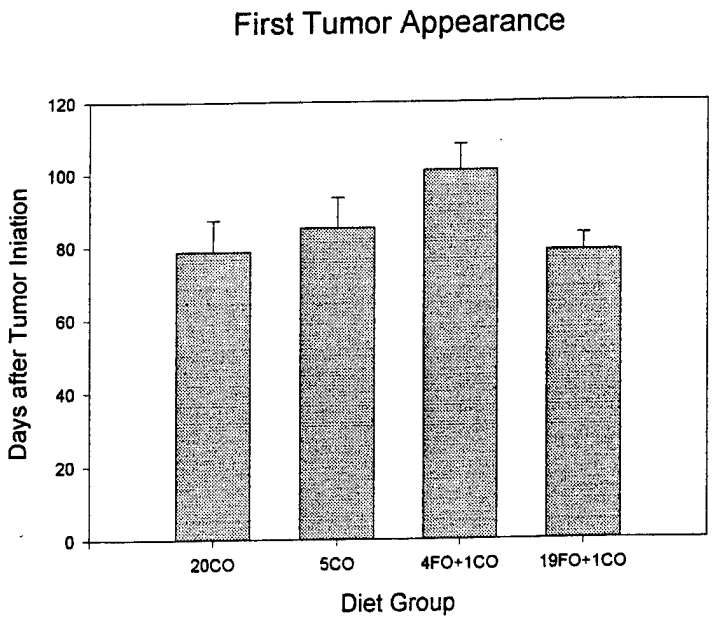
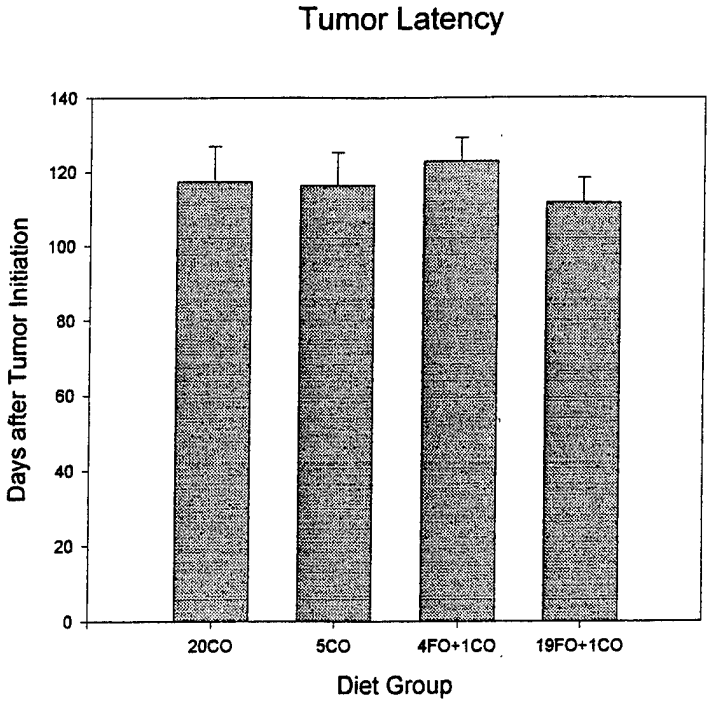


Figure 5.



Conclusions:

From this data, we believe we have demonstrated that our dietary interventions have resulted in correspondent changes in the fatty acid profiles of the tumors recovered from the different treatment groups. We were disappointed that the differences in some of the parameters were not as marked as in previous studies in our laboratory. The tumor development in the R3230AC transplant experiment did show a tendency for the 19%FO+1%CO group to have a reduced tumor burden, but the rapidity of the transplant growth and the small size of the groups made these differences less than statistically significant. The explanation as to why there were not more marked differences in tumorigenesis among the diet interventions in the NMU treated animals is difficult to interpret. In these animals there was a tendency towards both low fat groups having a delay in first tumor appearance, and there was some delay in the latency of the 4%FO+1%CO group. The 5%CO group had a statistically significant reduction in tumor burden and number of tumors per rat relative to the 20% CO group and the 4%FO+1%CO group showed a similar tendency. The reasons for the rats on the 19%FO+1%CO to not show the normally observed delay in tumor development is unclear. Whether this in any way related to the antioxidant status of the oils, or to some other unsuspected confounding factor is unclear. In June 1996 we initiated some additional studies to evaluate this more thoroughly. There was no evidence to suggest that the dietary fat alterations were associated with any significant change in serum levels of IGF. This is consistent with previous observations by our laboratory that the **in vitro** synthesis of growth hormone by pituitaries from rats on different quantitative omega-6 PUFA diets was not different despite significant differences in mammary tumor development. The studies assessing the nucleotide messages for the IGFs and **their** respective receptors in the tumor and its stroma will be carried out in 1997.